On the Nutritive Conditions Determining the Growth of certain Fresh-water and Soil Protista.

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[Plate 12.]

It is well known that in ponds and lakes cycles of development occur, in which various kinds of animals and plants replace one another in succession, but the conditions are usually so complex that the succession rarely repeats itself with regularity from year to year, and it is impossible to assign, with any certainty, the successive phases to their determining causes. The same kind of cyclical development occurs in artificially made organic infusions, where bacteria, algæ, flagellates and ciliates replace one another in irregular sequence.

The object of this paper is to indicate certain lines of experiment upon which it may be possible to attack this problem.

Woodruff has contributed some data for studying the underlying causes of these successive events, and the work of numerous authors has added to our knowledge of the factors regulating the growth of algæ and diatoms. Amongst these may be mentioned the work of Oswald Richter* on the nutrition of fresh-water algæ, and that of Miquel,† and more recently of Allen and Nelson,‡ on the culture of diatoms. The work of these authors tends to show that, even in the case of algæ and diatoms in which nutrition appears to be holophytic, the presence of some organic matter in the culture medium is of great assistance to the growth of the organisms.

The experiments with *Euglena viridis* were carried out with the object of investigating the nature of this organic matter which exerts a beneficial influence on the growth of apparently holophytic protista.

The method employed is to use a culture medium containing a constant proportion of the inorganic salts necessary for the nourishment of a holophytic organism, and to supply the organic matter in the form of

* Oswald Richter, 'Die Ernährung der Algen,' 1911.

† Miquel, 'Le Diatomiste,' 1892.

‡ E. J. Allen and E. W. Nelson, "On the Artificial Culture of Marine Plankton Organisms," 'Journ. Marine Biol. Assoc.,' vol. 8, No. 5, 1910.

chemically pure organic compounds instead of the indefinite composition of an organic infusion.

The method employed in the cultures of *Euglena viridis* has also been used to study the minute bacterial feeding flagellates living in the soil.

Experiments with Euglena viridis.

In culture experiments with *Euglena gracilis*, Zumstein* found that a much improved growth could be obtained if a little organic matter was added to the culture medium. By increasing the amount of organic matter in the medium, he found that *Euglena gracilis* could be induced to change its mode of nutrition, living solely as a saprophyte. Under these conditions the Euglena passed into an Astasia-like form, the chlorophyll disappearing and leaving only the colourless leucoplasts. When living saprophytically, the organism could thrive in the dark as well as in the light. Zumstein found that the green coloration was reassumed if the Astasia form was brought back into a solution containing only a small amount of organic matter and kept in the light.

Treboux† was able to grow *Euglena gracilis* in solutions containing citric acid, but found that *Euglena viridis* could not be grown under these conditions. Thus, there appears to be a marked physiological difference between these two species of Euglena, a fact which is emphasised by the earlier work of Klawkine‡ on *Euglena viridis*. His experiments showed that it was impossible to make *Euglena viridis* thrive well in the dark. When kept in the dark in a medium containing organic matter, the Euglenæ remained alive, but did not lose their chlorophyll or show a perceptible increase. Our experiments with this species of Euglena have confirmed the results obtained by Klawkine, and show that *Euglena viridis* is not able to thrive in the absence of light, even when placed in the optimal culture medium and in the presence of suitable organic matter.

It is thus evident that *Euglena viridis* is a more essentially holophytic organism than *Euglena gracilis*, a fact which tends to simplify the issue when we come to study the physiology of its nutrition.

By appropriate methods, a culture of *Euglena viridis* has been kept in active growth in test-tubes by inoculation from tube to tube, for a period of about two years.

* H. Zumstein, "Morphologie und Physiologie der *Euglena gracilis*," 'Pringsheim's Jahrbücher f. wiss. Botanik,' vol. 34, p. 419 (1899).

† O. Treboux, "Organische Säuren als Kohlenstoffquelle bei Algen," 'Ber. d. D. B. Ges.,' vol. 23, p. 432 (1905).

‡ W. Klawkine, "Recherches biologiques sur l'*Astasia ocellata* et l'*Euglena viridis*," 'Ann. des Sci. Nat., Zool.,' série 6, vol. 19, and série 7, vol. 1.

The medium used for growing these organisms has been a mixture of inorganic salts given by Miquel in his paper on the growth of diatoms.* To this medium, which contains all the elements necessary for the growth of a green plant, it has been found necessary to add some organic material in order to obtain an active growth of the organism. The composition of Miquel's fluid is as follows:—

Solution A.		Solution B.	
$MgSO_4$	10 grs.	Sodium phosphate.....	4 grs.
NaCl	10 „	Calcium chloride	4 „
Sodium sulphate	5 „	Hydrochloric acid	2 c.c.
Ammonium nitrate ...	1 „	Perchloride of iron, sat. sol.	2 „
Potassium nitrate	2 „	Water	80 „
Sodium nitrate	2 „		
Potassium bromide ...	0·2 „		
Potassium iodide	0·1 „		
Water	100 „		

To make up the fluid, 40 c.c. of solution A and 10 c.c. of solution B are added to 500 c.c. of tap water, and the mixture is filtered.

It was necessary at first to determine the strength of Miquel's fluid best adapted for growing the Euglena. In a number of preliminary experiments it was found that the best growth could be obtained when 4 c.c. of the above Miquel solution were added to 6 c.c. of tap water, the organic matter being supplied by 1 c.c. of hay infusion. The experiment was performed by inoculating the tubes with a very small amount of the stock culture, introduced by means of a capillary pipette. The tubes were kept in diffuse daylight at room temperature; attempts to hasten the growth by incubation at 75–80° F, were unsuccessful, the Euglena dying, or at any rate failing to flourish at this temperature. In the absence of any organic infusion, the Euglena either failed to develop or else multiplied very slowly, and the fluid in the tube never became crowded with free-swimming organisms so as to appear opaque and green. The addition of 1 or ½ c.c. of hay infusion, on the other hand, caused a thick growth which, after the lapse of 10–14 days, filled the tube with myriads of free-swimming individuals, giving a totally different appearance to the condition seen in the tubes to which no organic matter had been added.

It was found, however, that the efficacy of the hay infusion varied very greatly according to the length of time during which bacterial growth had continued in it. Thus, a fresh hay infusion, after being sterilised, was found

* Dr. Miquel, 'Le Diatomiste,' No. 9, June, 1892.

to have a much feebler effect in stimulating growth than an infusion which had been kept for some weeks and in which bacteria had been allowed to multiply before it was sterilised.

On the other hand, the same infusion, after being left for several months, lost much of its previous efficacy. Similar results were obtained with other vegetable infusions.

A typical experiment, showing the effect of the various dilutions of Miquel solution, and of the presence and absence of organic matter in the medium, may be seen in Table I.

Table I.—Cultures inoculated on April 21.

Tube No.	Composition of Medium.	Growth on April 29.	Growth on May 21.
1	10 c.c. Miquel solution	None	None
2	8 c.c. „ „ + 2 c.c. distilled H_2O	„	„
3	6 c.c. „ „ + 4 c.c. „ „	„	„
4	4 c.c. „ „ + 6 c.c. „ „	„	„
5	2 c.c. „ „ + 8 c.c. „ „	„	„
6	10 c.c. „ „ + 1 c.c. hay infusion	„	„
7	8 c.c. „ „ + 2 c.c. „ „ + 1 c.c. hay infusion	„	„
8	6 c.c. „ „ + 4 c.c. „ „ + 1 c.c. hay infusion	„	„
9	4 c.c. „ „ + 6 c.c. „ „ + 1 c.c. hay infusion	Slight growth	„
10	2 c.c. „ „ + 8 c.c. „ „ + 1 c.c. hay infusion	„	„
11	8 c.c. „ „ + 2 c.c. tap water	None	„
12	6 c.c. „ „ + 4 c.c. „	„	„
13	4 c.c. „ „ + 6 c.c. „	Slight growth	„
14	2 c.c. „ „ + 8 c.c. „	„	„
15	8 c.c. „ „ + 2 c.c. „ + 1 c.c. hay infusion	None	„
16	6 c.c. „ „ + 4 c.c. „ + 1 c.c. hay infusion	Slight growth	„
17	4 c.c. „ „ + 6 c.c. „ + 1 c.c. hay infusion	Very strong growth	Very strong growth
18	2 c.c. „ „ + 8 c.c. „ + 1 c.c. hay infusion	Strong growth	Good growth

These experiments show that the best growth was obtained in tube No. 17, in which the culture medium consisted of 4 c.c. Miquel solution + 6 c.c. tap water, to which 1 c.c. hay infusion was added; while in those tubes to which no organic matter was added, growth was either totally absent, or else a slight growth was observed in those tubes in which the proportions of Miquel and tap water approached the optimum.

It must be noted that when tap water is replaced by distilled water, the growth is either prevented altogether or else is very slight, even when the necessary elements for growth are given in the Miquel and organic matter. This can be seen in Table I in tubes Nos. 7–10, as compared with tubes Nos. 15–18. A similar result was noticed when cultures were made with Miquel fluid that had been made up with distilled water instead of tap water. The improved growth in tap water might be due either to the

difference in osmotic pressure or to the influence of some constituent, organic or inorganic, in the tap water.

An attempt was made to discover which of these was the determining influence. An artificial tap water was made up from an analysis of Thames water furnished us by Mr. W. W. Fisher. This contained :—

	Parts per 100,000.
$NaCl$	2·8
$NaNO_3$	0·7
$MgSO_4$	1·4
$CaSO_4$	2·8
$CaCO_3$	22·3
SiO_2	1·0
Total solids	31·0

Somewhat conflicting results were obtained when this artificial Thames water was used to replace natural tap water: on the whole the growth obtained was not so good as when natural tap water was employed, but since it was possible to obtain quite good growths with the artificial medium, it must be concluded that the superiority of the media containing tap water is due to some slight alteration in the proportions of the inorganic constituents.

Having determined the optimum conditions for growth as far as the inorganic constituents are concerned, namely, 4 c.c. of Miquel solution + 6 c.c. natural tap water, the question of the nature of the organic matter used by the Euglena was then taken up.

In the experiments given below, the ordinary method of inoculation by means of a capillary pipette was employed, and, in addition, another method which gives more rapid results. In this second method, an old culture tube, in which Euglena has been growing for a long period, is taken. In such a tube there is a ring of encysted Euglena adhering to the glass at the surface of the liquid. The liquid is poured away and the encysted Euglena, which is very firmly attached, may then be thoroughly washed with tap and distilled water. In this way a practically pure culture of Euglena may be obtained on adding the appropriate culture medium, though in no case has it been found possible to obtain a sterile culture free from bacterial contamination.

The following chemically pure substances were added to the "optimal Miquel" mixture and the tubes inoculated with Euglena, with the results subjoined.

1. *Dextrose*—

In media, in which 0·5–1 c.c. of a 1-per-cent. solution of dextrose was added to 10 c.c. of the "optimal Miquel" solution, no growth of Euglena was observed, but in all cases there was a considerable growth of fungus, probably derived from spores from the stock tube of Euglena. It is probable that the great development of the fungus inhibited the growth of the Euglena, since a slight growth in the optimal Miquel solution was expected.

2. *Cane Sugar*—

The addition of 0·5–1 c.c. of a 1-per-cent. solution of this substance to 10 c.c. of the "optimal Miquel" solution did not inhibit the growth of the Euglena to the same extent as the dextrose, but only rarely and after a long period did any noticeable growth appear. The growth of fungus in these tubes was either absent or very slight.

3. *Tartaric Acid*—

The addition of 1 c.c. of a 1-per-cent. solution had a purely negative effect, no growth of Euglena but a strong growth of fungus being observed.

It is evident from these results that the stimulating element in the organic infusion is not in the nature of a carbohydrate.

4. *Peptone*—

The addition of 1 c.c. of a 1-per-cent. solution of peptone to the medium invariably gave rise to a very strong bacterial growth, the bacteria being no doubt introduced with the Euglena, on inoculation. Under these conditions the Euglena scarcely developed at all, although it is not entirely killed off, a slight ring appearing at the top of the fluid.

5. *Amido-acids**—

Tyrosin.—As this compound is very insoluble in water, a saturated solution was made up in distilled water. The saturated solution when cold contains the salt in the proportion of 1 in 2400 of water.

In the earlier experiments 1 or 2 c.c. of the above solution were added to 10 c.c. of the "optimal Miquel" mixture. A very strong growth was obtained in this medium, indeed superior to that obtained by means of the addition of a natural organic infusion. The marked difference in the growth of the Euglena in a tube containing this minute trace of tyrosin (1 : 24,000) as compared with a culture in a medium free from organic matter, may be seen in the photograph (fig. 1).

* A number of experiments have been made by Loew, Bokorny, and others on the growth of algæ in amido and fatty acids. A full literature of this work will be found in Oswald Richter's 'Die Ernährung der Algen,' 1911.

In cultures containing 1–2 c.c. of the tyrosin solution, it was found that after a period of about six to eight weeks the Euglena ceased its active growth and became encysted upon the walls of the tube and especially round the surface. For example, a tube containing 4 c.c. Miquel solution, 6 c.c. tap water, and 1 c.c. tyrosin solution, was inoculated with Euglena on November 25. By December 3 there was a noticeable growth, which became very thick by December 30. By January 30 all free-swimming forms had disappeared, and nothing remained but a ring of encysted

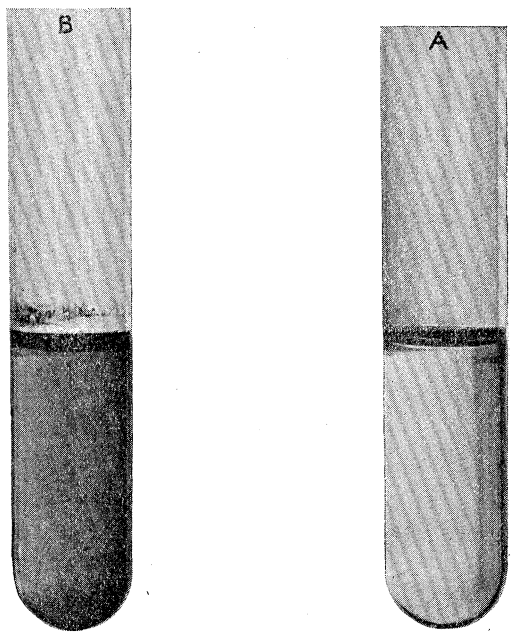


FIG. 1.—Photograph to show the Growth of Euglena in a Tube (B), containing the optimal Miquel mixture, to which tyrosin solution was added, as described.

Tube A shows the slight growth in a control culture containing the optimal Miquel solution, but with no organic solution.

Photograph shows a 10-days growth.

Euglena. It was found that by replenishing the culture medium in this tube, the growth of the Euglena could at once be revived: within two days after replenishment a thick growth of free-swimming forms was obtained.

This suggested that the tyrosin was used up after a certain period. To test this hypothesis, cultures were made in the optimal Miquel mixture, to which tyrosin was added in the solid form, so that as soon as the dissolved tyrosin was used up, fresh tyrosin might go into solution. Cultures grown in this medium showed a very rapid growth of Euglena during the first fortnight or three weeks, but after that the increased development of

bacteria in the culture usually interferes with the Euglena growth. The following culture may be regarded as typical of the growth of Euglena in a medium of this nature:—

Cultures with Tyrosin Media, inoculated February 2.

Composition of tube.	Growth on Feb. 5.	Growth on Feb. 18.
4 c.c. Miquel tap + 6 c.c. tap water + solid tyrosin	Very strong Euglena growth.	Euglena dead or encysted; numerous bacteria.
4 c.c. Miquel tap + 6 c.c. tap water + 1 c.c. tyrosin solution	Slight growth of Euglena.	Very strong Euglena growth; very few bacteria.

To avoid the excessive growth of bacteria in the tyrosin and at the same time to ensure the continuous supply of tyrosin, the following culture method was devised. The Euglena was grown in a tube containing the optimal Miquel mixture alone, and the trace of tyrosin was supplied from another tube containing a saturated solution of this substance connected by means of a capillary tube with the Euglena culture. In this way the culture medium is continually supplied with traces of tyrosin solution, but the diffusion is too slow to cause an excess of tyrosin in the tube containing the Euglena. It was found that by this method a strong growth of Euglena could gradually be obtained, nearly free from the bacteria and minute flagellates which always appeared in cultures to which solid tyrosin was added.

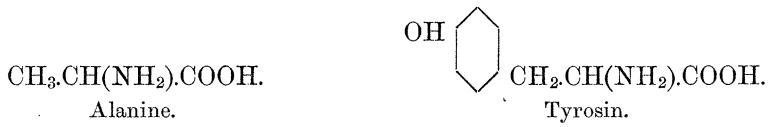
Of all the culture media employed the thickest and most successful growths of Euglena have been obtained with optimal Miquel mixture to which tyrosin is added.

Glycocoll.—Cultures were made in optimal Miquel mixture to which was added 1 c.c. of 1-per-cent. solution of glycocoll. These cultures invariably gave a strong growth of bacteria and, at first, a greatly retarded growth of Euglena, though subsequently the Euglena increased. In no case did these cultures compare in strength of growth with the cultures in tyrosin media. It is probable that this retardation was due to the bacterial growth, and this subject will be dealt with in the latter part of the paper.

Alanine.—1 c.c. of a 1-per-cent. alanine solution was added to the Miquel mixture as usual. The cultures invariably gave a very strong bacterial growth, and very frequently a bacillus producing a vivid apple-green coloration appeared. This green colouring matter was shown not to be chlorophyll, as it was developed more rapidly and to a greater degree in the dark than in the light. At first, as in glycocoll, the Euglena failed to multiply, though after a long period, viz. about three weeks, tubes inoculated

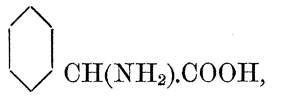
with a ring of encysted forms, as described above, produced a considerable growth.

The great superiority of the tyrosin solutions over the solutions of glycocoll and alanine was very marked. It was at first thought possible that this was due to the presence of the benzene ring in the tyrosin, especially since alanine is similar in composition to tyrosin, except that in the former substance the oxyphenyl ring is absent.



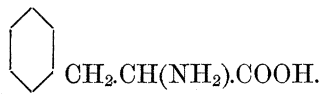
With a view to testing this hypothesis, the phenyl compounds of alanine and glycocoll were employed in the culture media.

In media containing phenyl glycocoll,



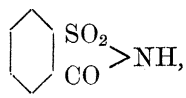
it was found that no growth took place, even the development of the bacteria being prevented.

But, in the media containing phenylalanine,



a very strong growth of Euglena was produced, bacterial growth being at first slight, but increasing after some time. Since this compound resembles tyrosin in being very insoluble, it was added to the media in a solid form.

Attempts to grow Euglena in saccharin,



showed that this substance prohibited all growth of the organism.

The negative results obtained with phenyl glycocoll and with saccharin showed that, at any rate, the mere presence of the benzene ring was not the essential factor for the growth of the Euglena.

Since the substances that are most successful for the propagation of Euglena, namely, tyrosin and phenylalanine, are only very slightly soluble, so that exceedingly weak solutions are used, and since, on this account, bacterial growth in these solutions is very slight compared with that which occurs in the stronger solutions of alanine and glycin, it seemed possible

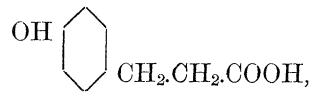
that the strong growth of the Euglena might be connected with the slight bacterial growth.

To test this hypothesis, lesser amounts of alanine and glycocoll were added to the Miquel mixture.

From 0·2 c.c. to 0·5 c.c. were added to 10 c.c. of the optimal Miquel mixture. In cultures started from a ring of encysted forms it was found possible to obtain extremely good growths of Euglena by this means, the bacterial growth being very much lessened.

It is thus obvious that the Euglena can use alanine and glycocoll, as well as tyrosin and phenylalanine, provided its growth is not inhibited by the rapid development of bacteria, such as always takes place in the glycocoll and alanine solutions when they are too strong. This result is of importance as indicating that the amido-acids are used as such by the Euglena, and not after being decomposed by bacterial growth. Thus, attempts made to grow Euglena in tubes containing an alanine medium in which bacterial decomposition had proceeded for a long time were entirely unsuccessful.

It is interesting to notice in this connection the fact mentioned above that, in the case of tyrosin, the addition of the solid substance to the tubes causes a considerable bacterial growth, which, after about three weeks, was sufficient to inhibit the proper growth of the Euglena. Nencki* has shown that, under the influence of anaërobic bacteria, tyrosin is converted into oxyphenylpropionic acid,



and it is probable that, under the aërobic conditions met with in the culture tubes, further decomposition into oxyphenylacetic acid and phenol takes place.

Decomposition along similar lines occurs when phenylalanine is subjected to bacterial growth, phenylpropionic acid and phenylacetic acid being formed. Thus, when tyrosin and phenylalanine are added in the solid condition, their solutions are sufficiently strong to allow a growth of bacteria, which decompose them into phenol derivatives that are harmful to the Euglena growth.

We may suppose that, in the same way, harmful products are produced by bacterial action on alanine and glycocoll, so that the Euglena is prevented from developing in the solutions of a strength adapted to the growth of bacteria.

Other nitrogenous compounds have been tried, *e.g.* urea, uric acid, and

* Nencki, 'Ber. d. Deutsch. Chem. Gesellsch.,' 1874, p. 1593.

allantoin. All these substances gave negative results, and no growth of Euglena could be obtained in optimal Miquel mixture to which these substances were added.

Thus, no substances other than compounds of the amido-acid type, have been found suitable for stimulating the growth of the Euglena.

If we enquire into the part played by the amido-acids in the nutrition of Euglena, it may first be noted that the Euglena is obtaining the greater part of its nutriment from the CO_2 of the air and from the mineral substances in the Miquel mixture. This was readily proved by keeping a control tube, containing the optimal Miquel solution to which tyrosin had been added, in the dark, in which case the growth of the Euglena was at once arrested (fig. 2).

It must also be pointed out that the amount of amido-acid present in the optimal culture medium is exceedingly minute; *e.g.* in the case of tyrosin

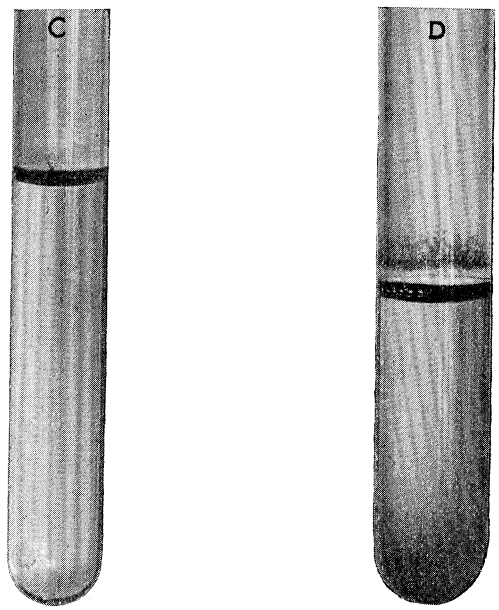


FIG. 2.—Photograph showing the Growth of Euglena in Miquel Mixture and Tyrosin, in the dark (tube C), and in the light (tube D).
Photographed three weeks after inoculation.

solution the amount of the salt is only 1 part in 24,000 of liquid. It is very remarkable that so minute a trace of organic matter can make so great a difference in the rapidity of growth and reproduction in an organism as shown in the first photograph (fig. 1). It would appear that the organic substance acts more as a stimulant than as a direct source of nutriment.

The facts observed in the culture of Euglena may be summarised as follows:—

(*a*) In solutions containing no organic matter, the Euglena increases very slowly.

(*b*) By the addition of a trace of organic infusion to the solution of inorganic salts, a good growth of Euglena can often be obtained.

(*c*) The efficacy of the natural organic infusion in stimulating the growth was very variable.

(*d*) Minute traces of amido-acids added to the inorganic solution had a remarkable effect in stimulating the growth of the Euglena.

(*e*) Stronger solutions of amido-acids were less successful owing to the rapid development of bacteria in the medium.

(*f*) The Euglena does not appear to live saprophytically on the amido-acid, since it cannot be made to thrive in the absence of light.

2. *Experiments with Soil Protozoa.**

The method of growing Protozoa in solutions containing a mixture of Miquel in tap water to which various organic compounds are added was also applied with a view to studying the protozoal fauna of various soils.

The mode of procedure was similar to that employed in the experiments on Euglena. The cultures were made in sterilised test-tubes to which the optimal Miquel solution was added, the solutions also being carefully sterilised. Various organic solutions were added to the various tubes, which were inoculated by adding a small amount of soil to each tube. This method was found to be particularly suited to the culture of the minute soil flagellates, more especially *Prowazekia terricola* described by Martin.†

The following Table shows a typical series of cultures conducted as described above:—

* See Dr. Russel and Dr. Hutchison, "On the Effect of Partial Sterilisation of Soil on the Production of Plant Food," 'Journ. Agric. Sci.,' vol. 3, part 2 (1909); also Goodey, 'Roy. Soc. Proc.,' B, vol. 84, p. 165 (1911).

† C. H. Martin, 'Zool. Anzeiger,' vol. 41, No. 10 (1913). A flagellate monad, similar to that described by C. H. Martin ('Roy. Soc. Proc.,' B, vol. 85, 1912), was found in small numbers in our cultures.

Composition.	Inoculation, March 8.	Observations on March 13.
4 c.c. Miquel tap + 6 c.c. tap—		
1. + 1 c.c. cane sugar solution	Controls. Not inoculated	Few bacteria only.
2. + solid tyrosin		Sterile.
3. + solid phenylalanine		Sterile.
4. + 1 c.c. cane sugar	Inoculated with stale manure	A few soil flagellates. Some ciliates.
5. + solid tyrosin		Very large numbers of soil flagellates and of soil amœbæ.
6. + solid phenylalanine		Large numbers of flagellates; a few ciliates.
7. + 1 c.c. cane sugar	Inoculated with leaf mould	A few soil flagellates.
8. + solid tyrosin		Very large numbers of flagellates.
9. + solid phenylalanine		Large numbers of flagellates.
10. + 1 c.c. cane sugar	Inoculated with ploughed soil	Very few flagellates.
11. + solid tyrosin		No flagellates.
12. + solid phenylalanine		Very few flagellates.
13. + 1 c.c. cane sugar	Inoculated with soil under grass land.	No flagellates.
14. + solid tyrosin		Very few flagellates.
15. + solid phenylalanine		Fair number of flagellates.

The above Table illustrates the fact that while the minute soil flagellates thrive best in tyrosin or in phenylalanine solutions, yet they are able to develop in solutions containing cane sugar. Cultures were made with the object of ascertaining the effect of various other organic substances on the growth of the flagellates. The flagellates in these cultures were derived for the most part from a stock tube of Euglena culture in tyrosin, in which Prowazekia was also very abundant. The following list embodies the results obtained with various organic substances. Save where otherwise mentioned, the organic compounds were added in the proportion of 1 c.c. of a 1-per-cent. solution to 10 c.c. of the optimal Miquel mixture in tap water.

	Growth of the flagellates.
Peptone	Good growth.
Tyrosin ($OH.C_6H_5.CH_2.CH(NH_2).COOH$)	Very strong growth.
Tyrosin (added solid)	Optimum growth.
Phenylalanine, $C_6H_5.CH_2.CH(NH_2)COOH$ (added solid)	Strong growth.
Alanine $CH_3.CH(NH_2).COOH$ (0·5 c.c. of 1-per-cent. solution)	Fair growth.
Glycocoll, $CH_2(NH_2).COOH$	Fair growth.
Phenylglycocoll, $C_6H_5.CH(NH_2).COOH$	No growth.
Allantoin	No growth.
Saccharin	No growth.
Cane sugar	Good growth.
Tartaric acid	No growth.

These cultures showed that the soil flagellates were able to grow in tubes containing a large variety of organic substances, in many of which Euglena is

unable to thrive. This is the result of the holozoic mode of nutrition of the flagellates, which feed greedily on the bacteria in the culture and are always to be found in greatest abundance in the bacterial scum at the surface.

The development of the soil flagellates in the culture is evidently dependent upon the bacterial flora in the tube. In the tyrosin media the bacterial growth reaches its most favourable degree for the development of the flagellates. In the case of cultures in media containing alanine it is frequently found that the flagellates fail to attain their maximum growth, being probably swamped by the excessive numbers of bacteria.

In order to discover whether the Miquel salts were necessary for the growth of the soil flagellates, a culture medium was made up by adding solid tyrosin to 10 c.c. of tap-water, and this was inoculated with a strong culture of *Prowazekia terricola*. This culture entirely failed to develop, remaining almost entirely free even of bacteria, which were evidently unable to develop satisfactorily in the absence of the salts of the Miquel solution.

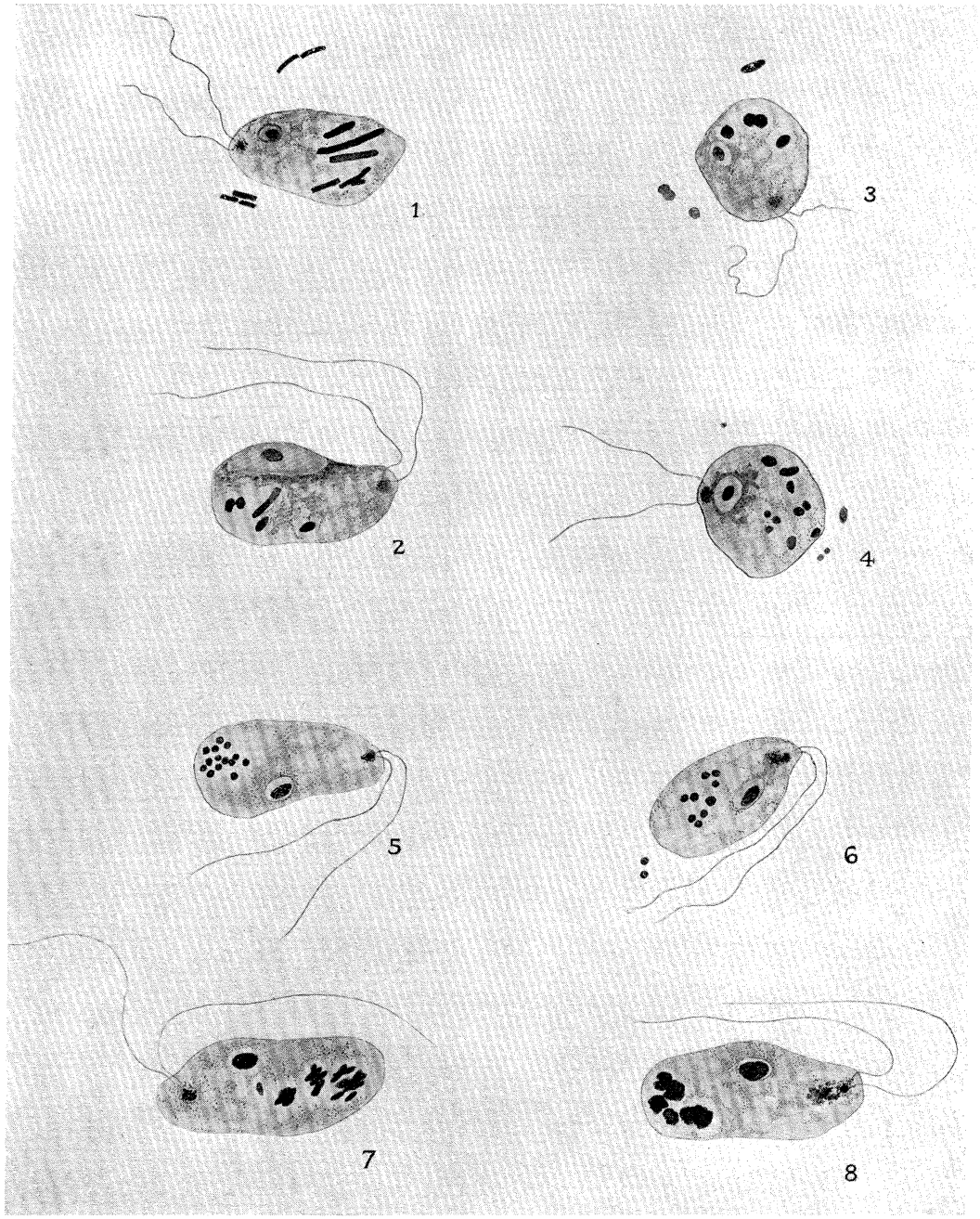
The Prowazekia were observed to flourish in cultures containing very varied types of bacteria. In order to discover whether the flagellates exercised any selective faculty when feeding upon the bacteria, a number of smear preparations were made from different cultures. The films were fixed with corrosive acetic or with osmic vapour, and were stained with iron hæmatoxylin. These preparations showed that bacteria of all the types surrounding the flagellates were ingested in quite a promiscuous manner (see Plate 12).

The cultures inoculated with various soils, both in the test-tubes and in drop cultures which were also made, show the enormous abundance and wide distribution of these minute flagellates as compared with other soil protozoa.

Although ciliates and amœbæ often fail to appear in tubes inoculated with a very small quantity of soil, yet all the types of soil that have been tried have yielded at least some Prowazekia when inoculated into the appropriate culture media. The organism has also been found in tap water and in water from an open-air tank.

The very rapid increase of these minute flagellates is also very noticeable. Under the optimum culture conditions it has been found possible to obtain a strong growth of the flagellates within 48 hours of the time of inoculation. On the other hand the larger protozoa, such as the ciliates, do not become even noticeable in the tubes until a week or so has elapsed.

The great abundance and wide distribution of the minute flagellates, taken in conjunction with their rapid powers of increase, suggest that in all probability they are of much greater importance than the larger soil protozoa as a factor in the destruction of soil bacteria.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The ease with which Prowazekia can be grown in culture media containing tyrosin suggests the possibility of investigating its distribution in various types of soil. Experiments in this direction are at present very incomplete, but as far as they go they tend to show that rich manure soil or leaf mould contains a considerably greater number of the minute flagellates than less rich soils. This is well seen in Table II. (Compare Nos. 4–9 with Nos. 10–15.)

In summing up the points observed in the cultures of soil flagellates we notice the following facts:—

(*a*) As compared with Euglena they are able to live in cultures to which organic compounds of very varying natures have been added.

(*b*) This comparative impartiality is the result of the holozoic mode of nutrition, the development of the flagellates being absolutely dependent on the bacterial growth.

(*c*) The presence of the Miquel salts in the solution is necessary for the growth of the soil flagellates and for the proper development of the bacteria upon which they feed.

(*d*) The flagellates can feed upon a variety of different types of bacteria.

DESCRIPTION OF PLATE.

Soil Flagellates from a Culture containing a Mixed Bacterial Flora, showing various Types of Ingested Bacteria. × 2000.

Figs. 1–2.—Two individuals containing ingested bacilli.
„ 3–6.—Individuals containing cocci of two kinds.
„ 7–8.—Two individuals containing partially digested bacteria.

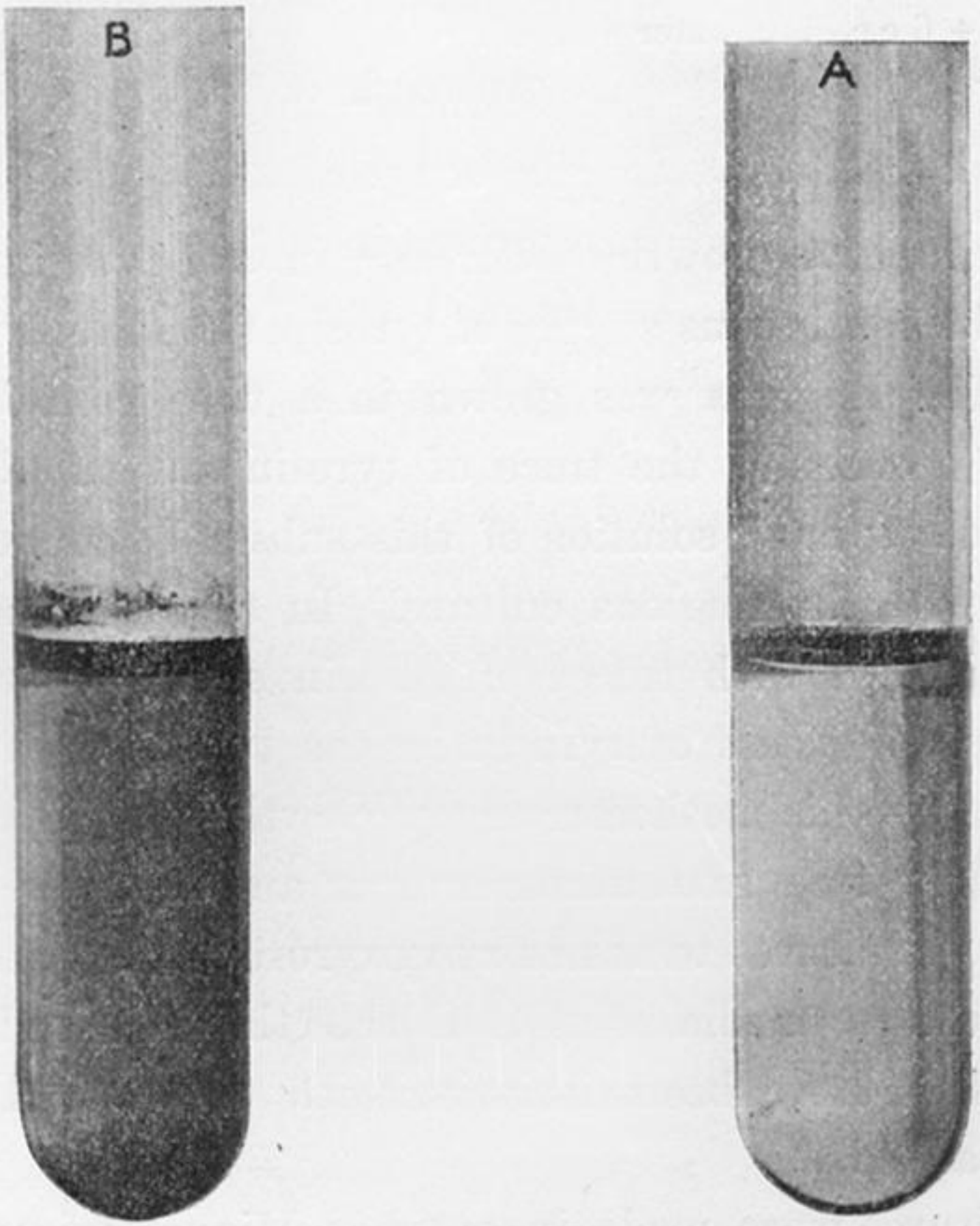


FIG. 1.—Photograph to show the Growth of Euglena in a Tube (B), containing the optimal Miquel mixture, to which tyrosin solution was added, as described.

Tube A shows the slight growth in a control culture containing the optimal Miquel solution, but with no organic solution.

Photograph shows a 10-days growth.

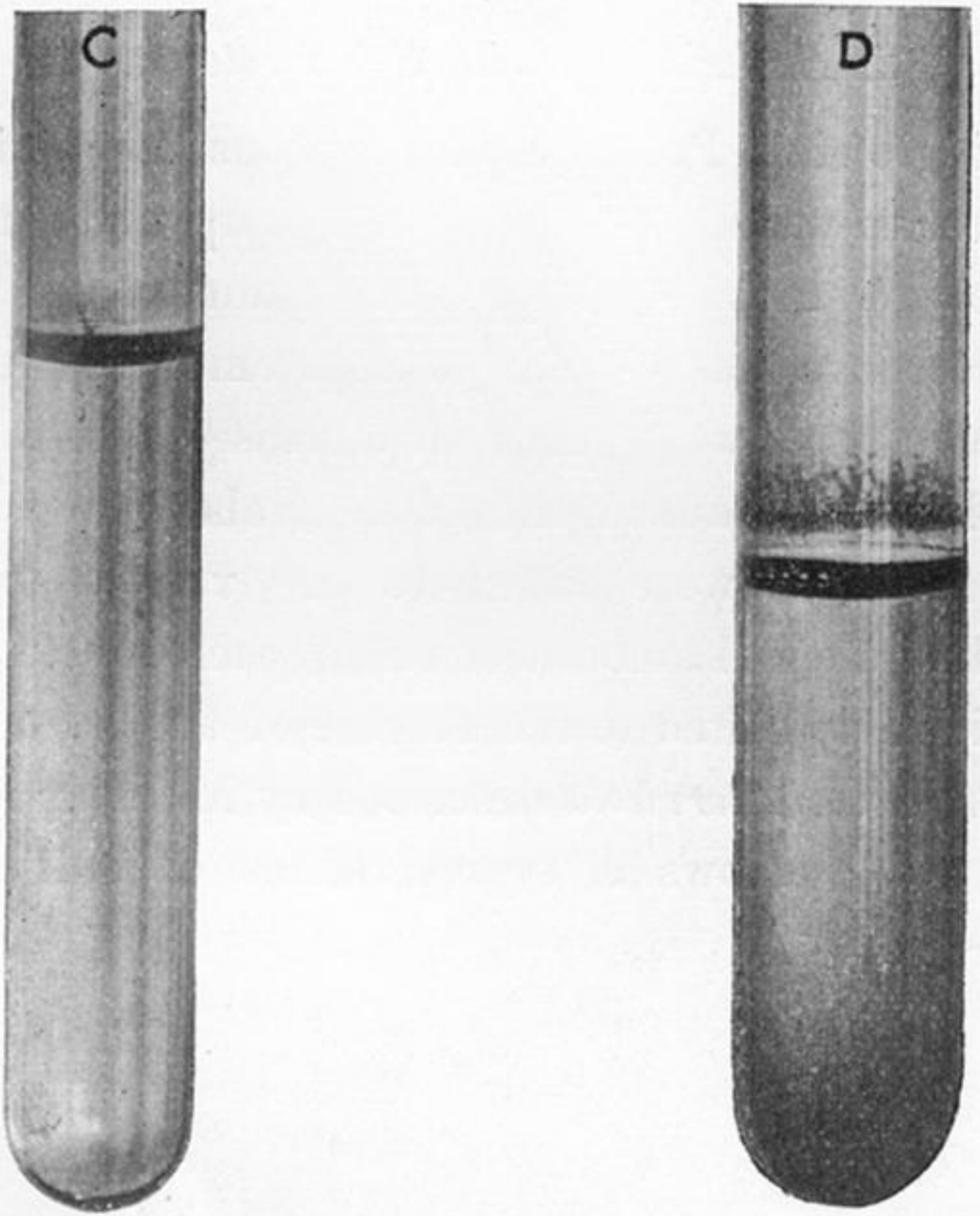
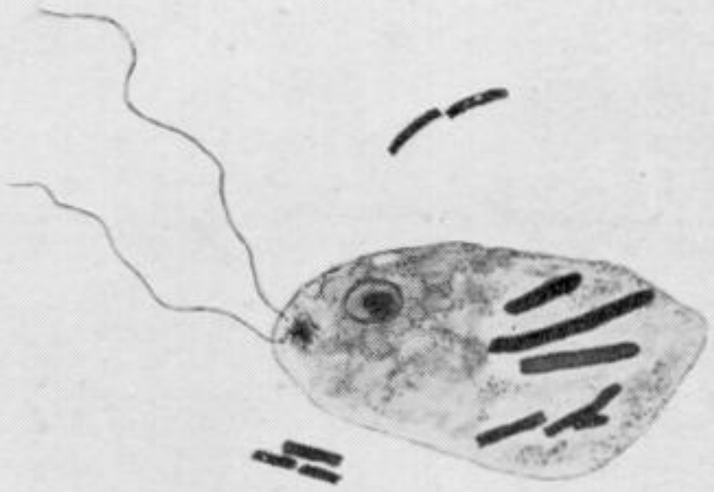


FIG. 2.—Photograph showing the Growth of Euglena in Miquel Mixture and Tyrosin, in the dark (tube C), and in the light (tube D).

Photographed three weeks after inoculation.



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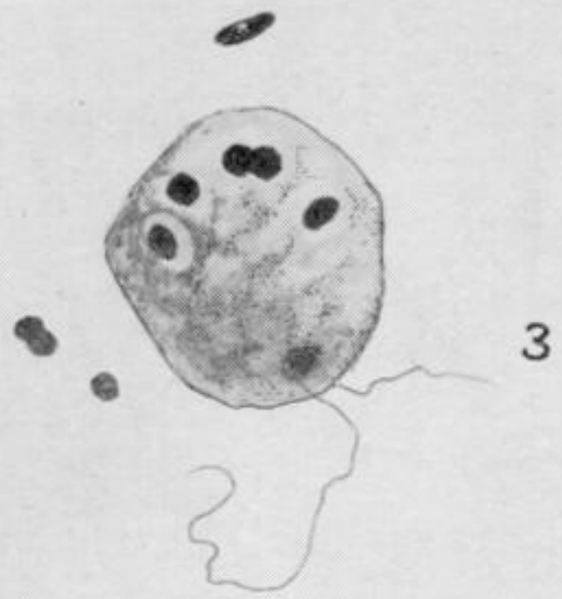

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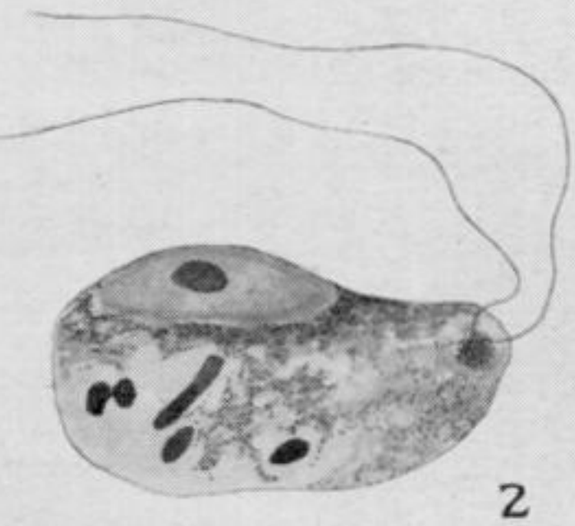

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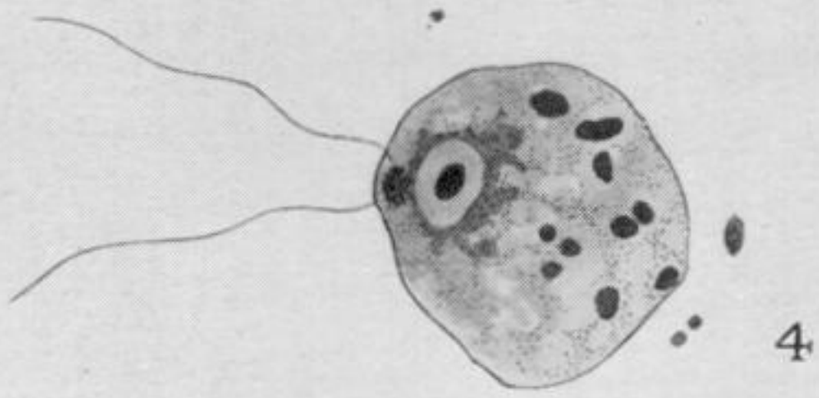

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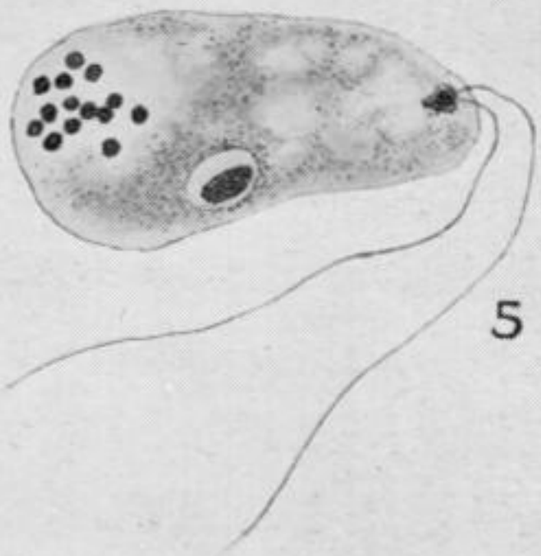

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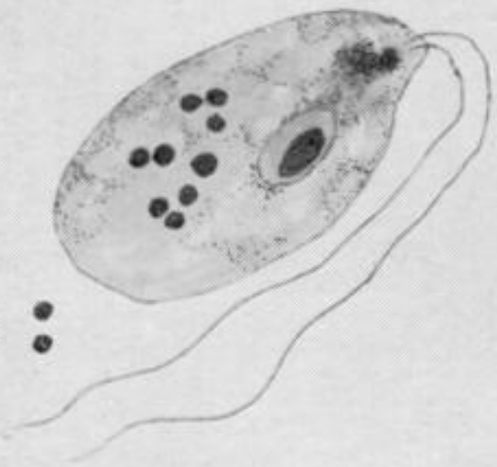

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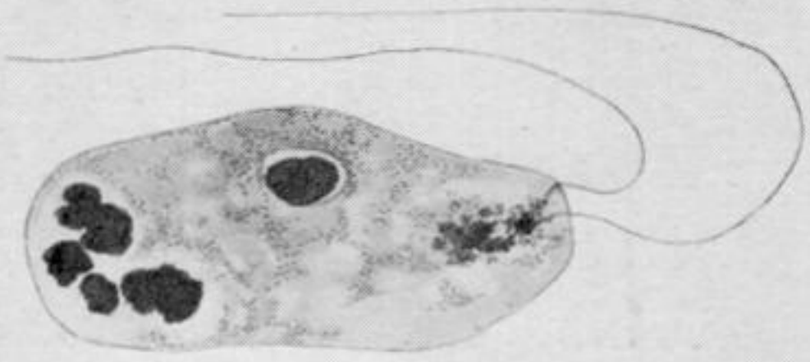

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